Detection of CYP1A2 in Formalin-Fixed, Paraffin-Embedded Rat Tissue

Reagent and Antibody Information

1X Wash Buffer
3% Hydrogen Peroxide
1% BSA Diluent
1X Citrate Buffer
DAB Chromogen
Hematoxylin

Blocking Serum: Normal Rabbit Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog # 011-000-001

Avidin / Biotin Blocking Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # SP-2001

Primary Antibody: Sheep Anti-Rat Cytochrome P450 CYP1A2 Antibody
Millipore
Billerica, Massachusetts 01821
www.millipore.com
1-800-645-5476
Catalog # AB1249

Negative Control Serum: Normal Sheep Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog # 013-000-001

Secondary Antibody: Rabbit Anti-Sheep IgG (H+L) Biotin-SP-Conjugated
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog # 313-065-003

Label Complex: Peroxidase-Conjugated Streptavidin SS Label
Biogenex Laboratories
San Ramon, CA 94583
www.biogenex.com
1-800-421-4149
Catalog # HK330-9K

Staining Procedure

Positive Control Tissue: Liver (upregulated by treatment)
Stain Localization: Cytoplasmic (centrilobular staining pattern)

1. Deparaffinize and hydrate slides through the following solutions:

Solution	Repetitions	Time
Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X Wash Buffer	2 times	5 minutes

2. Quench endogenous peroxidase by placing the slides in 3% hydrogen peroxide for 15 minutes.

3. Rinse slides in 2 changes of 1X wash buffer for 5 minutes each.

4. Heat-Induced Epitope Retrieval Using The Microwave
   Place a full rack of slides into a Tissue Tek® container with 200 ml of 1X citrate buffer
   (Insert blank slides into any empty slots in the rack to ensure even heating of slides)
   Microwave for 5 minutes at power level 5.
   Cool for 1 minute. (Add more citrate buffer, if necessary.)
   Microwave again for 5 minutes at power level 5. *Temperature Before Cooling Slides*_____
   Cool 20 minutes at room temperature.
   Rinse the slides in 2 changes of distilled water for 3 minutes each time.

5. Block with 10% normal rabbit serum for 20 minutes at room temperature.
   Lot #_____ Date Reconstituted_____

   DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

6. Avidin / Biotin Blocking Kit
   Lot #_____ Exp. Date_____ New Kit: yes / no
   Apply avidin block for 15 minutes at room temperature.
   Quick rinse in 1X wash buffer.
   Apply biotin block for 15 minutes at room temperature.

   DO NOT RINSE SECTIONS WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.
   ONLY WIPE EXCESS BLOCK.

7. Apply primary antibody at a 1:400 dilution. Incubate for 1 hour at room temperature.
   Lot #_____ Exp. Date _____

For negative control slides, dilute normal sheep control serum so that it's protein concentration matches that of the primary antibody. Then make a 1:400 dilution. If the concentrations can't be matched using this method, the dilution for the negative reagent may need to be adjusted. Apply the negative and incubate for 1 hour at room temperature.
Lot #_____ Exp. Date _____

8. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each time.

9. Apply the rabbit anti-sheep secondary antibody at a 1:500 dilution. Incubate for 30 minutes at room temperature.
   Lot #_____ Date Reconstituted_____

10. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each time.

11. Apply the Streptavidin SS Label. Incubate for 30 minutes at room temperature.
    Lot #_____ Exp. Date _____

12. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

13. Apply the DAB chromogen. Incubate in the dark for 6 minutes at room temperature.
    (Add 1 drop of DAB per ml of substrate)
    Lot #_____ Exp. Date_____ New Kit: yes / no

14. Rinse the slides in tap water 3 minutes.

15. Counterstain with hematoxylin for 20 seconds.

16. Rinse the slides in tap water until water is clear.

17. Gently agitate slides in 1X wash buffer until the tissues turn blue.

18. Dehydrate through the following solutions:

Solutions	Repetitions	Time
95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

19. Coverslip

Updated 02/01/06